Recent Advances in Synchrotron-Based Microscopy

A Joint Workshop of the Advanced Light Source and Stanford Synchrotron Radiation Laboratory at the ALS October 7-8, 2003

Organizing Committee: Greg Denbeaux (University at Albany), Khatherina Luening (SSRL), Gary Mitchell (Dow Chemical), Piero Piannetta (SSRL) and David Shuh (LBL)

Tuesday October 7, 2003

User Meeting continues all morning.

1:25 PM Opening Comments

Spectro-Microscopic Data Interpretation / New instrumental capabilities

1:30 Adam Hitchcock (Brockhouse Institute for Materials Research, McMaster University)
Methods and examples of quantitative chemical mapping by soft X-ray spectromicroscopy

2:00 Chris Jacobson (Dept. Physics & Astronomy, Stony Brook University)
Cluster analysis for soft x-ray spectromicroscopy

2:30 David Kilcoyne (ALS)
Interferometrically controlled microscopes at 5.3.2 and 11

3:00 Tolek, Tyliszczak (ALS)
New instrumental capabilities at MES Beamline 11

3:30 Break

Applications to environmental Science

3:45 John Lawrence (NHRC)
Comparative scanning transmission x-ray and laser scanning microscopy of microbial biofilms

4:10 Klaus Pecher (Pacific Northwest National Laboratory)
Applications of Scanning Transmission X-ray Spectromicroscopy (STXM) for the characterization of transition element precipitates in environmental science

4:35 Alain Manceau (CNRS)

5:00 Adjourn

Recent Advances in Synchrotron-Based Microscopy

Wednesday October 8, 2003

Applications to soft matter

9:00 Rainer Fink (Univ. Erlangen-Nuernberg, Physikalische Chemie II)
Spectroscopy of Organic Nanocrystals Using the ALS-STXMs

9:25 Stephen Urquhart (U. of Sask.)
Linear Dichroism and Imaging of Polycrystalline Alkanes

9:50 George Cody (Geophysical Laboratory, Carngegie Institution of Washington)
Probing degradation chemistry in the cellular membrane of vascular plants: Biogeochemical applications of STXM

10:15 Break

10:30 Ying Zou (Department of Physics, North Carolina State University)
Growth of short chain alkanes and polyethylene in crystalline and semi-crystalline thin films

10:55 Torhu Araki (Depts. of Chemistry[1] & Pediatrics[2], McMaster University)
Quantitative chemical mapping of polymer reinforcement of alginate microcapsules by soft X-ray spectromicroscopy

11:20 Chang Chang (Drexel University)
New Optical Designs for High Spatial and Spectral Resolution X-Ray Microscope

11:45 Carolyn Larabell (LBL/UCSF)
X-ray Tomography of Cells

12:10 Lunch Provided by ALS

Applications to magnetic materials and Hard X-ray microscopy

1:30 Peter Fischer (Max-Planck)
High resolution magnetic imaging with full-field soft X-ray microscopy

1:55 Sug-Bong Choe
Time and Spatially Resolved Microscopy on Magnetization Dynamics Using X-PEEM

2:20 Katharina Luening (SSRL)
A hard x-ray imaging facility at SPEAR3

2:45 Break

3:00 Wenbing Yun (Xradia, Incorporated)
Imaging biological specimens with hard x-rays: advantages and challenges

3:25 Barry Lai (APS)
X-ray fluorescence microscopy and microspectroscopy for biological applications

3:50 Ulrich Neuhaeusler (ESRF)

Zernike-type phase contrast X-ray microscopy at 4 keV photon energy with 60 nm spatial resolution

4:20 **Eduardo Almeida** (NASA Ames Research Center and University of California San Francisco)
X-Ray Microscopy - Filling the Gap between Light and Electron Microscopy in the Biological Sciences

4:45 **Adjourn**

Abstracts
Tuesday October 7, 2003

1:30 PM **Adam Hitchcock** (Brockhouse Institute for Materials Research, McMaster University)

Methods and examples of quantitative chemical mapping by soft X-ray spectromicroscopy

Soft X-ray spectromicroscopy (a.k.a. NEXAFS microscopy) is a powerful tool for quantitative chemical analysis. Due to the ability of soft X-rays to penetrate water, and on account of lower radiation damage than electron beam techniques, scanning transmission X-ray microscopy (STXM) is an ideal tool to study soft matter such as polymer, biological, and environmental systems. The near edge X-ray absorption fine structure (NEXAFS) signal provides the basis for detailed, quantitative speciation. I will outline procedures available in the aXis2000 package to derive quantitative maps and illustrate these methods with examples from STXM and PEEM studies of biofilms for environmental remediation, protein adsorption on patterned polymers, and analysis of industrial samples. The capabilities and pitfalls of presently implemented methods will be outlined and challenges, along with potential opportunities for further development of analysis modalities, will be discussed.

Measurements performed at 5.3.2 STXM and 7.3.1 PEEM at the Advanced Light Source, which is funded by DoE under contract DE-AC03-76SF00098. Research is supported by Dow Chemical, Ricoh, NSERC (Canada) and the Canada Research Chair program.

2:00 PM **Chris Jacobson** (Dept. Physics & Astronomy, Stony Brook University)

Cluster analysis for soft x-ray spectromicroscopy

Soft x-ray spectromicroscopy provides spectral data on the chemical speciation of light elements at sub-100 nanometer spatial resolution. When all chemical species in a specimen are known and separately characterized, existing approaches can be used to measure the concentration of each component at each pixel. In other cases (such as often occur in biology or environmental science), where the specimen may be too complicated or provide at least some unknown spectral signatures, other approaches must be used. We describe here an approach that uses principal component analysis (also called factor analysis) to orthogonalize and noise-filter spectromicroscopy data. We then use cluster analysis or pattern matching to classify pixels according to spectral similarity, extract representative, cluster-averaged spectra with good signal-to-noise ratio, and obtain gradations of concentration of these representative spectra at each pixel in the data. The

method is illustrated with a simulated data set of organic compounds, and a mixture of lutetium in hematite used to understand colloidal transport properties of radionuclides.

2:30 PM **David Kilcoyne** (ALS)
Interferometrically controlled microscopes at 5.3.2 and 7-11

3:00 PM **Tolek, Tyliszczak** (ALS)
New instrumental capabilities at 7-11

3:30 PM Break

Applications to environmental Science

3:45 PM **John Lawrence J.R. Lawrence[1*], T. Araki[2], S. Zhang[2], M.M. West[3], G. D.W. Swerhone[1], G.G. Leppard[4], T.R. Neu[5], and A.P. Hitchcock[2]**
[1] National Water Research Institute, 11 Innovation Blvd., Saskatoon, SK, Canada S7N 3H5 [2]-Department of Chemistry and Brockhouse Institute for Materials Research, McMaster University, Hamilton, Ontario, Canada L8S 4M1 [3]Electron Microscopy Facility, Faculty of Health Sciences, McMaster University, 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5 [4]NWRI, PO Box 5050, 867 Lakeshore Road, Burlington, Ontario, Canada L7R 4A6 [5]UFZ Centre for Environmental Research, Magdeburg, Germany.

Comparative scanning transmission x-ray and laser scanning microscopy of microbial biofilms

Confocal laser scanning microscopy (CLSM) and soft x-ray scanning transmission x-ray microscopy (STXM) were used to map the distribution of macromolecular sub components (polysaccharides, proteins, lipids, nucleic acids) and associated elements of the biofilm cells and matrix. STXM uses the intrinsic x-ray absorption properties of the sample eliminating the need for addition of reflective, absorptive, or fluorescent probes and markers relied upon in CLSM and which may introduce artefacts or complicate interpretation. Specific C 1s reference spectra were developed for use in the analyses. The biofilms were developed from river water supplemented with methanol and, although a complex microbial community, were dominated by heterotrophic bacteria. STXM and CLSM measurements were performed on a wet cell constructed from films grown directly on a silicon nitride window mounted on biofilm reactor strips. CLSM provided detailed compositional information when used in conjunction with molecular probes, and STXM provided compositional mapping of macromolecule distributions without addition of probes. Results from various edges detected in STXM and probe defined areas of CLSM were used to map the distribution of nucleic acids, protein, carbohydrate and lipid in the biofilm community, along with that of Ni relative to these components. Both approaches indicated that intra-cellular protein and nucleic acids were present, although the exopolymer matrix was clearly dominated by polysaccharides. Elemental analyses of

nickel in the biofilm showed a large degree of metal ion concentration (more than 1000-fold) with a very selective distribution. By examining exactly the same region of a sample with combinations of these techniques we demonstrate that both probe dependent and probe independent methods may be used to map microbial biofilm structure, composition and associated elements.

4:10 PM **Klaus Pecher** (PNNL)

Applications of Scanning Transmission X-ray Spectromicroscopy (STXM) for the characterization of transition element precipitates in environmental science

The interfaces between solid mineral particles and water play a crucial role in partitioning and chemical transformation of many inorganic as well as organic pollutants in environmental systems. Among environmentally significant minerals, mixed-valent oxides and hydroxides of iron (e.g. magnetite, green rusts) and manganese bioprecipitates have been recognized as particularly strong sorbents for metal ions and as redox active compounds for environmental pollutants such as Cr(VI), U(VI), phenols, and halogenated hydrocarbons. STXM not only provides information about the morphology of fully hydrated particles on a submicron scale, but also can give chemical state information by spatially resolved XANES (X-ray Absorption Near Edge Structure). It therefore has recently attracted interest as a characterization tool for applications in environmental nano-science, where the shrinking particle size of transition metal oxides is expected to extend their range of physical properties.

4:35 PM **Alain Manceau** (CNRS)

5:00 PM **Adjourn**

Wednesday October 8, 2003

Applications to soft matter

9:00 AM **Rainer Fink** (Univ. Erlangen-Nuernberg, Physikalische Chemie II)

Spectroscopy of Organic Nanocrystals Using the ALS-STXMs

Near-edge x-ray absorption fine structure is well-suited to investigate the electronic structure of organic substances. We have used the ALS polymer STXM to study several classes of organic materials: heteroaromatic monomers, metal-organic or pure organic charge-transfer complexes and molecular nanomagnets. Common to most of these studies is, that the spatial resolution on a sub-100 nm scale is absolutely necessary to correlate the spectral properties with crystalline phases of the respective material, in particular when the films are prepared from solution.

9:25 AM **Stephen Urquhart** (U. of Sask.)

Linear Dichroism and Imaging of Polycrystalline Alkanes

9:50 AM **George Cody** (Geophysical Laboratory, Carngegie Institution of Washington)

Probing degradation chemistry in the cellular membrane of vascular plants: Biogeochemical applications of STXM

10:15 AM **Break**

10:30 AM Y. Zou[(a)], A.D.L. Kilcoyne[(a)], H. Ade[(a)], Y. Wang[(b)], M. Rafailovich[(b)]

[(a)] Department of Physics, North Carolina State University
[(b)] Department of Materials Science and Engineering, State University of New York at Stony Brook

Growth of short chain alkanes and polyethylene in crystalline and semi-crystalline thin films

Scanning transmission x-ray microscopy (STXM) has been used to determine the three fundamental dichroism spectra of crystalline n-tetracontane ($C_{40}H_{82}$) corresponding to the three directions of the orthorombic unit cell of crystalline polyethylene. Based on linear dichroisms in NEXAFS spectra, the orientation of long molecular chains in single crystals of solvent cast tetracontane are found to be perpendicular to a Si_3N_4 membrane surface. The two anisotropic lateral crystalline axis a, b can be distinguished by intensity changes in the spectral doublet near 287eV and associated with the orientation of the unit

cell of tetracontane single crystals. Prior NEXAFS dichroism data of PE and alkanes integrated over the a and b directions and only the dichroism between the C-C chain along the c-axis and the average for the C-H bonds have been known. This new information helps us further to understand the growth orientation of polyethylene macromolecules in ultrathin films from the melt. Preliminary STXM data suggests that in spherulitic-type growth in the thin film, the C-C bonds are in the plane of the thin film and perpendicular to the fibrils. Furthermore, in very thin films, the average NEXAFS spectrum normal to the film switches from a slight dominance of b-axis signal to one of slight a-axis signal dominance. This implies that in very thin films, the interfacial constraints alter the average orientations of crystallites. The same average spectra are found for PE thin film as in the bulk for PE of the same density, clearly indicating that PE thin films are just as crystalline in thin films as they are in the bulk.

10:55 AM **T. Araki[1], X. Zhang[1], A.P. Hitchcock[1], F.Shen[2], P.Chang[2], M.Wang[1], and R. Childs[1]**

Depts. of Chemistry[1] & Pediatrics[2], McMaster University, Hamilton, ON, CANADA

Quantitative chemical mapping of polymer reinforcement of alginate microcapsules by soft X-ray spectromicroscopy

The mechanical and chemical properties of alginate capsules can be modified to improve their performance as immuno-isolating devices for gene therapy. These modifications consist of the addition and in situ photo-polymerization of sodium acrylate and N-vinylpyrrolidone in the alginate capsule to form additional covalent cross-links. We have used scanning transmission X-ray microscopy (STXM) in the regions of C1s, N1s, O1s, and Ca 2p absorption edges at beamline 5.3.2 to probe the structure of the modified capsules. Analysis of X-ray image sequences and selected area spectra mapped the calcium gradient in capsules, identified the presence of polyacrylate throughout the capsules and poly-N-vinylpyrrolidone in the outer regions of the alginate capsules. These quantitative maps of their spatial distributions have led to further understanding of the chemical modifications that produce a mechanically more stable capsule structure. Challenges in this project relating to sample preparation, radiation damage, and analysis of dilute components will be discussed

11:20 AM **Chang Chang** (Drexel University)

New Optical Designs for High Spatial and Spectral Resolution X-Ray Microscope

High spatial and spectral resolution microscopes operating in the soft x-ray wavelength region have long been desired. Currently, two complementary types of x-ray microscope, full-field x-ray microscope and scanning x-ray microscope, have been developed. A brief comparison will be presented, along with an assessment on the feasibility of high spatial and spectral resolution undulator-based x-ray microscope.

11:45 AM **Carolyn Larabell** (LBL/UCSF)

X-ray Tomography of Cells

12:10 PM **Lunch** Provided by ALS

Applications to magnetic materials and Hard X-ray microscopy

1:30 PM **Peter Fischer** (Max-Planck)

High resolution magnetic imaging with full-field soft X-ray microscopy

A fundamental understanding of magnetism on a nm length and sub-ns time scale is currently a focus of basic and applied solid state physics research. The origin of interlayer exchange coupling, perpendicular magnetic anisotropies, unusual magneto resistance effects, magnetisation reversal mechanisms and dynamical effects such as precessional and domain wall motions and damping/relaxation phenomena are only a few examples. Low dimensional magnetic systems with lateral patterning are also promising candidates in future magnetic storage and sensor technologies, where both the miniaturisation and the speeding up of switching processes are crucial. The combination of XMCD with full field soft X-ray transmission microscopy (magnetic transmission X-ray microscopy (MTXM) allows for an element-specific imaging of magnetic domain structures with a lateral resolution at 20nm. In particular the recording in varying external magnetic fields allows for detailed studies of nucleation and reversal mechanism. Using the pulsed time structure of 3rd generation synchrotrons with stroboscopic pump-and-probe techniques one is in addition now able to image the magnetisation dynamics on a sub-ns time scale. I shall review the current status and the future perspectives of MTXM with emphasis on the capabilites at the ALS that will contribute substantially to current issues in the field of nanomagnetism.

1:55 PM **Sug-Bong Choe[1], Yves Acremann[2], Andreas Bauer[1,2,3], Andreas Scholl[1], Andrew Doran[1], Joachim Stöhr[2], Howard A. Padmore[1]**

[1] Advanced Light Source [2] Stanford Synchrotron Radiation Laboratory [3] Freie Universität Berlin, Arnimallee 14, D-14195 Berlin, Germany

Time and Spatially Resolved Microscopy on Magnetization Dynamics Using X-PEEM

Understanding magnetization dynamics in external magnetic fields on the ps time scale is essential for future high performance magneto-electronic devices, since the state of the art technology is limited to switching time of about 1 ns. Below 1 ns the magnetic response is determined by the fundamental processes of spin precession and frictional damping whose microscopic origins are not well understood. Utilizing the high spatial

resolution Photoemission Electron Microscope PEEM-2 at the ALS and the intrinsic time resolution given by the ALS pulse width, we have successfully observed magnetization dynamics on the 100 ps time scale and 100 nm length scale. The time resolution was achieved by utilizing a pump-probe technique with fs laser pump for short magnetic pulse generation and ALS synchrotron x-ray probe for x-ray microscopy observation. Magnetic field pulse was created by current pulse, launched from a photoconductive switch through a lithographically produced waveguide structure. The switch was triggered by a fs laser, synchronized to the pulse train of the ALS, operated in two bunch mode. The pulse profile was directly determined by utilizing the deflection of images that arised from the deflection of the measured electron signal due to the additional electric field on the waveguide during the current pulse. The fast evolution of magnetic domains was monitored as a function of the delay between the magnetic field pulse (pump) and the x-ray pulse (probe) using a stroboscopic pump-probe setup. Dynamics of magnetic vortex, a flux closure state of a curling magnetization, was directly observed for the first time and will be reported here as a proof of principle.

2:20 PM **Katharina Luening[1], Piero Pianetta[1],**
Wenbing Yun[2], Jonathan Trent[3], Eduardo Almeida[3]
[1]SSRL, [2]Xradia Incorporated, [3]NASA Ames Research Center

Full Field hard x-ray microscopy at SSRL

The Stanford Synchrotron Radiation Laboratory (SSRL) in collaboration with Xradia Inc. and the NASA Ames Research Center is going to implement a hard x-ray full field imaging microscope on a Wiggler beam line at SPEAR3. This facility will provide unprecedented analytical capabilities for a broad range of scientific areas and will emphasize research on nanoscale phenomena and structures in materials science, environmental science, and biology. The instrument itself will be a commercial, full-field transmission microscope (TXM) based on zone plate optics from Xradia, Inc. This instrument will enable high resolution x-ray microscopy, tomography, and spectromicroscopy in a photon energy range between 3–14 keV. The spatial resolution of the TXM microscope is specified as 60 nm for imaging in the first order diffraction of the micro zone plate and 20 nm in third diffraction order. It will be shown that this imaging facility will optimally combine the latest imaging technology developed by Xradia Inc. with the wiggler source characteristics at beam line 6-2 of SSRL. This will result in an instrument capable of high speed and high resolution imaging with spectral tunability for spectromicroscopy, element specific and Zernicke phase contrast imaging. Furthermore, a scanning microprobe capability will be integral to the system thus allowing elemental mapping and fluorescence yield XANES to be performed with a spatial resolution of 1 μm without introducing any changes to the optical configuration of the microscope.

2:45 PM **Break**

3:00 PM **Wenbing Yun** (Xradia, Incorporated)

Imaging biological specimens with hard x-rays: advantages and challenges

3:25 PM **B. Lai, S. Vogt, J. Maser, D. Legnini, Z. Cai**

Advanced Photon Source, Argonne National Laboratory, Argonne, IL 60439

X-ray fluorescence microscopy and microspectroscopy for biological applications

The importance of metals in biological and medical sciences has received increased recognition. The metal may be involved in part of a physiological or pathogenic process (metalloproteins, cellular ions, etc.), or it may be introduced externally (environmental contaminants, metal-based drugs, etc.). Since cellular concentrations of metal are typically very low, part-per-million or less, x-ray fluorescence microscopy is ideally suited for studying trace metal distribution due to its inherent low background. It enables quantitative studies of extra- and intra-cellular distributions of elements from Si to Zn and above, with sub-optical spatial resolution. Additionally, the possibility to select the incident X-ray energy with a bandwidth of $\Delta E/E = 10^{-4}$ enables microspectroscopy and chemical state mapping to determine the speciation of elements of interest. These unique capabilities of x-ray fluorescence microscopy complement other modern microscopy techniques and thus has been employed in biomedical applications with single cells [1-3] and bacteria [4]. We will discuss instrumentation and methods that have been implemented, demonstrate their application in several ongoing collaborations, and delineate the future prospects.

[1] C.T. Dillon et al., J. Biol. Inorg. Chem. **7**, 640 (2002).
[2] T. Paunesku et al., Nature Materials **2**, 343 (2003).
[3] P. Ilinski et al., Cancer Research **63**, 1776 (2003).
[4] M. Labrenz et al., Science **290** (2000) 1744.

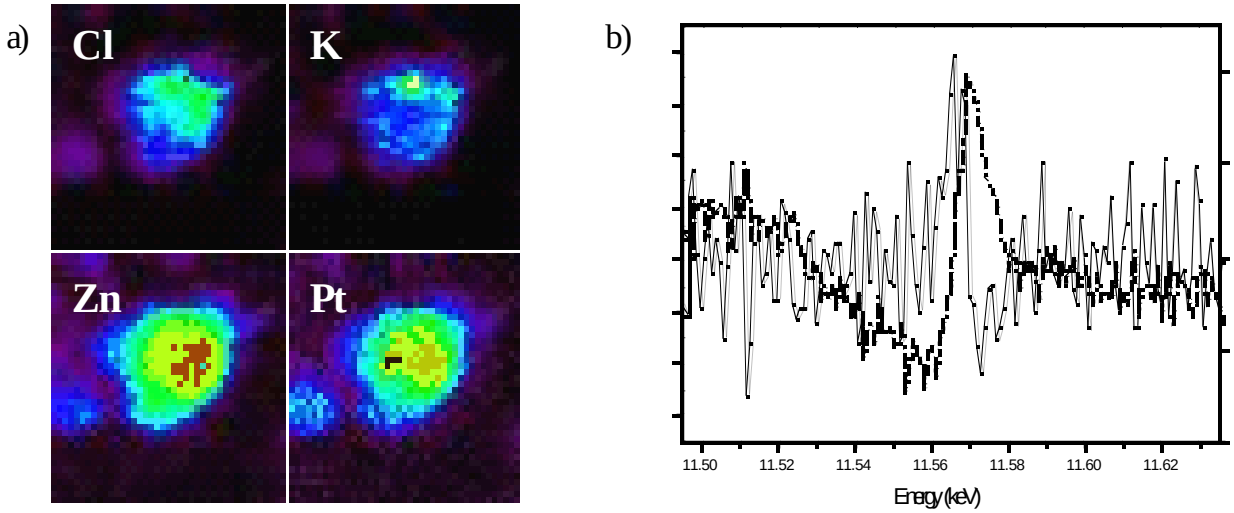

Fig. 1. a) Fluorescence maps, and b) micro-XANES at the Pt L_{III} edge, for a A2780 ovarian cancer cell treated with platinum(IV) complexes.

3:50 PM **Ulrich Neuhaeusler** (ESRF)

Zernike-type phase contrast X-ray microscopy at 4 keV photon energy with 60 nm spatial resolution

X-ray microscopy in the multi-keV photon energy range offers unique possibilities to study relatively (10-50 µm) thick dense samples with high spatial resolution. When employing a high N.A. condenser zone plate sample illumination in combination with a high resolution micro zone plate objective lens, a spatial resolution of currently 60 nm is achieved in the full-field transmission X-ray microscope setup at ID21 beamline of the ESRF, operating at 4 keV photon energy.

Since the absorption becomes smaller with increasing photon energy, phase contrast imaging allows one to overcome the limitation for imaging weakly absorbing structures in amplitude contrast mode, because the real part of the refractive index (representing phase shift) drops less drastically than the imaginary part (representing absorption).

We will report on X-ray microscopy of advanced microelectronic devices imaged using the developed Zernike phase contrast microscopy technique. While the amplitude contrast between copper and silicon dioxide in these samples is only 7 %, negative as well as positive phase contrast were demonstrated with a big enhancement of contrast to 40 % and 45 %, respectively.

We will briefly discuss other concepts of zone plate based phase contrast X-ray microscopy techniques, summarize the current status and discuss the outlook for potential future developments of this field.

4:20 PM Eduardo Almeida (NASA Ames Research Center and University of California San Francisco)

X-Ray Microscopy - Filling the Gap between Light and Electron Microscopy in the Biological Sciences

Conventional light microscopy, including immunofluorescence microscopy has been the main tool biologists have used to visualize cellular localization of specific proteins and cellular structures since the advent of modern cell biology. Although electron microscopy has been widely used for structural studies, cell biologists have not been able to effectively take advantage of its high resolution for functional studies of the cell. Recent developments in X-ray microscopy may now allow us to examine conventional light and immunofluorescence microscopy samples at a resolution about one order of magnitude better than light microscopy, while retaining the ability to immunolabel specific proteins or structures. Potential techniques for observation include immunometal labeling, density and phase contrasts imaging, as well as use of contrast enhancing metals such as osmium. X-ray microscopy has the potential to fill an important gap of visualization of the nanoscale molecular machinery of the cell in a novel way that has not been possible with light and electron microscopy.

4:45 PM Adjourn